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Genome Analysis

Gas vesicles in actinomycetes: old buoys in novel habitats?

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Gas vesicles are gas-filled prokaryotic organelles that function as flotation devices. This enables planktonic cyanobacteria and halophilic archaea to position themselves within the water column to make optimal use of light and nutrients. Few terrestrial microbes are known to contain gas vesicles. Genome sequences that have become available recently for many bacteria from non-planktonic habitats reveal gas vesicle gene clusters in members of the actinomycete genera *Streptomyces, Frankia* and *Rhodococcus,* which typically live in soils and sediments. Remarkably, there is an additional level of complexity in cluster number and gene content. Here, we discuss whether putative gas vesicle proteins in these actinomycetes might actually be involved in flotation or whether they might fulfil other cellular functions.

Introduction

Gas vesicles are gas-filled organelles that have been studied extensively in cyanobacteria and halophilic archaea (for a review see [1]). The gas vesicle envelope consists of an amphipathic protein membrane with a ribbed ultrastructure, and comprises minimally seven proteins [2]. These gas vesicle proteins (Gvps) are encoded by a cluster of *gvp* genes (Figure 1). Early gene disruption experiments indicated that 13 *gvp* genes are essential for wild-type gas vesicle formation in halophilic archaea [3], whereas the findings of a more recent study suggest that eight genes, *gvpFGJKLM-gvpAO*, suffice [4]. GvpA represents the major envelope component. Two sets of paralogous proteins, the GvpA paralogues GvpJ and GvpM and the paralogous GvpF and GvpL proteins, are also found in archaeal gas vesicles [2]. The function of GvpG, -K and -O is still unclear; however, GvpC provides rigidity to the vesicle but is not essential for vesicle integrity. Moreover, GvpC has been suggested to shape gas vesicles [5–8].

Intriguingly, orthologues of the eight essential *gvp* genes occur in duplicate or even triplicate in the genomes of the saprophytic soil bacteria *Streptomyces coelicolor* and *Streptomyces avermitilis* (Figure 1) [4,9,10]. These organisms are developmentally complex, mycelial members of the actinomycetes, which are high-GC Gram-positive bacteria with a remarkable capacity to produce a vast array of secondary metabolites, including many antibiotics. The unexpected discovery of *gvp* gene clusters in the genomes of several actinomycetes raises interesting questions concerning their functions.

gvp gene clusters in *Streptomyces* species

The *gvp* clusters of *S. coelicolor* and *S. avermitilis* have a similar gene order: *gvpO, A, F, G, J, L, S, K* (Figure 1). In

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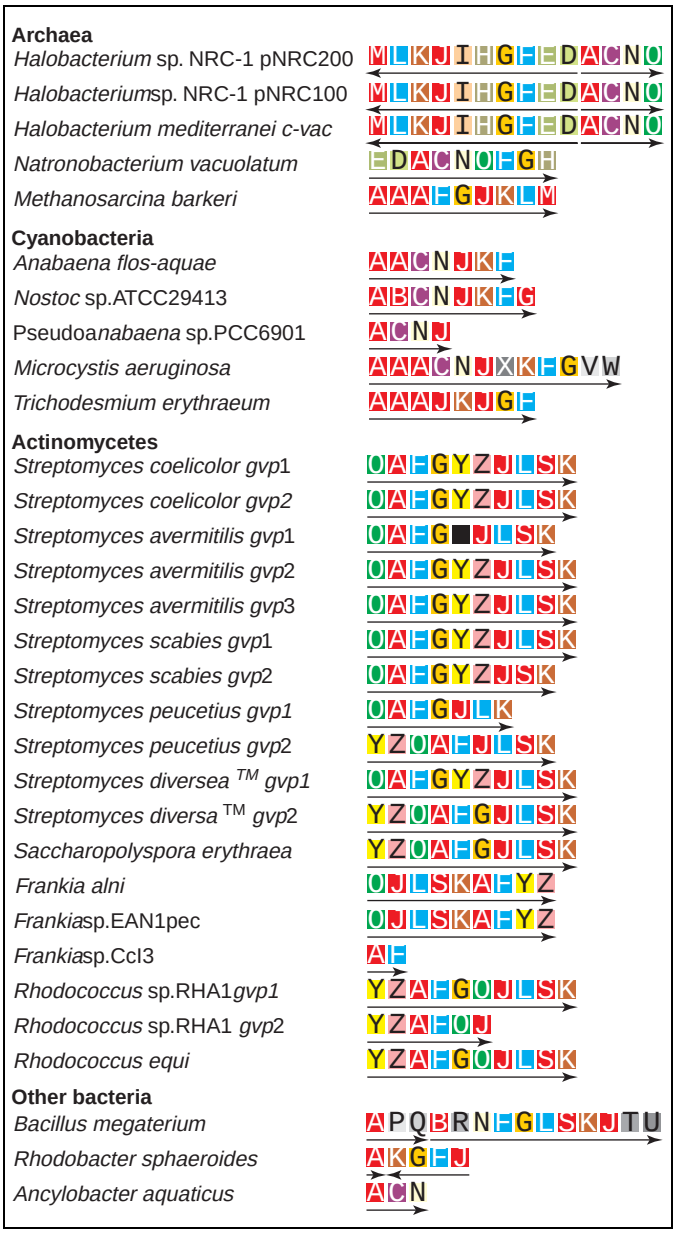

Figure 1. Organization of *gvp* gene clusters. Each letter identifies a *gvp* gene. Orthologous genes, and paralogues in the same cluster, are in identical colours. Transcription direction is indicated by arrows.

four of these five clusters, two novel genes – *gvpY* and *Z* – lie between *gvpG* and *J*, whereas, in *gvp1* of *S. avermitilis*, these are replaced by a single open reading frame (ORF). None of the clusters encodes GvpC, which is required for gas vesicles to resist higher pressures, particularly in deep lakes [5].

Recently, genome-sequencing projects have revealed pairs of *gvp* clusters in three other *Streptomyces* species: the plant pathogen *S. scabies* (http://www.sanger.ac.uk/Projects/S_scabies); the adriamycin-producer *S. peucetius* (J.K. Sohng, personal communication); and '*S. diversa*™', a derivative of *S. venezuelae* engineered for enhanced secondary metabolism (M.J. Bibb, personal communication). Of these, *gvp1* in *S. scabies* and '*S. diversa*™' resembles the *S. coelicolor* and *S. avermitilis* clusters, whereas the others differ in the presence and position of *gvpYZ* or in lacking *gvpL* (*S. scabies gvp2*), *gvpS* (*S. peucetius gvp1*) or *gvpG* (*S. peucetius gvp2*).

Gas vesicle genes in other actinomycetes

Several other soil actinomycetes also contain *gvp* clusters (Figure 1). Whether these organisms actually synthesize gas vesicles remains to be established; nevertheless, the filamentous erythromycin-producer *Saccharopolyspora erythraea* (P. Leadlay, personal communication) and the filamentous balhimycin-producer *Amycolatopsis balhimycina* (W. Wohlleben and T. Weber, personal communication) both have at least one *gvp* cluster. The unicellular animal pathogen *Rhodococcus equi* (http://www.sanger.ac.uk/Projects/R_equi/) and the filamentous PCB-degrader, *Rhodococcus* sp. RHA1 (http://www.rhodococcus.ca/), both contain a *gvp* cluster. Perhaps most surprising is the discovery of *gvp* genes in *Frankia* spp., which are filamentous N_2-fixing actinomycetes that live symbiotically in actinorhizal root nodules of shrubs and trees (P. Normand, personal communication; http://genome.jgi-psf.org/mic_home.html). Interestingly, *Frankia* sp. Ccl3, with the smallest genome and only a limited host-range, also contains the smallest *gvp* gene cluster, comprising only *gvpAF* (Figure 1). It is noteworthy that the sequenced mycobacteria and corynebacteria, with smaller genomes than most other actinomycetes, lack *gvp* genes (http://www.ncbi.nlm.nih.gov/; http://www.sanger.ac.uk/Projects/Pathogens/).

Actinomycete GvpA proteins have a C-terminal extension

An amino acid sequence alignment of various putative GvpA proteins revealed a long C-terminal extension on actinomycete GvpAs but not on other GvpAs (Figure 2). This extension almost doubles the length of actinomycete GvpA proteins and does not resemble other known sequences. Moreover, this additional domain has extraordinarily high levels of glutamate and arginine, and to a lesser extent proline, which are often present in long, alternating acidic and basic tracts. Epitope display experiments imply that the C terminus of GvpA might be situated on the cytosolic side of the gas vesicle in *Halobacterium* sp. NRC-1 [11,12] and the unusual properties of actinomycete C-terminal GvpA could hint towards alternative functions for gas vesicles in actinomycetes.

The N-terminal half of actinomycete GvpAs aligns well with the full-length GvpAs of non-actinomycetes. This enabled construction of an unrooted phylogenetic tree (Figure 3). Cyanobacterial, archaeal and actinomycete GvpAs each form a distinct clade. GvpA of *Streptomyces* and *Frankia* spp. formed separate subclades within the actinomycete clade. GvpA2 of *S. coelicolor* and GvpA3 of *S. avermitilis* formed a deep-branching group within the *Streptomyces* sub-clade. Surprisingly, GvpA of *Sac. erythraea* displayed particularly deep-branching, grouping most closely with GvpA of rhodococci as a third subclade.

The separate actinomycete GvpA branch indicates that these *gvp* genes were not acquired by lateral gene transfer. Furthermore, a global comparison of the genomes of *S. coelicolor* and *Frankia alni* (P. Normand, personal communication) showed significant overall synteny including *gvp* of *F. alni* and *gvp1* of *S. coelicolor*. This

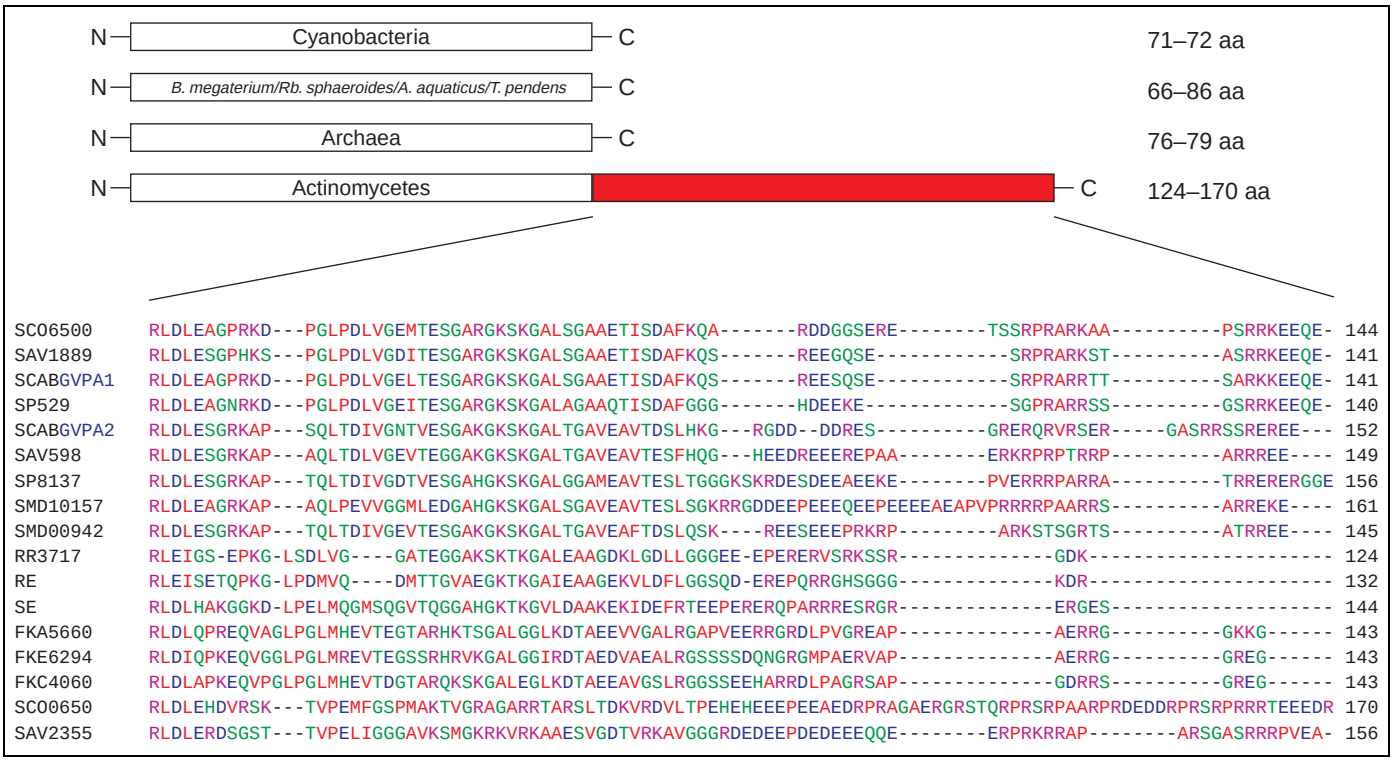

Figure 2. Domain organization of GvpA showing extraordinary length and content of the C termini of actinomycete GvpA proteins. Amino acid (aa) numbers indicate the full length of proteins.

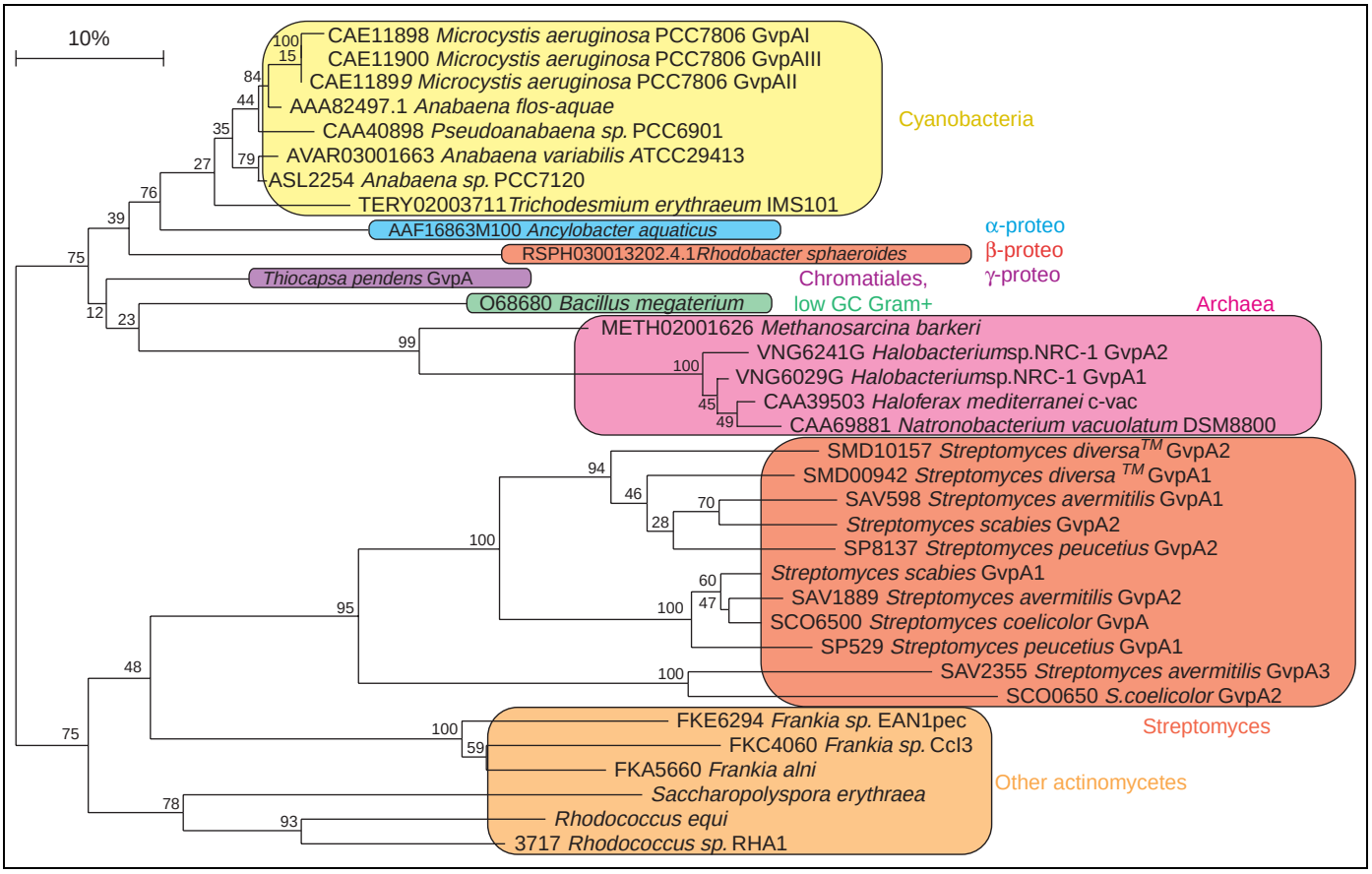

Figure 3. Phylogenetic relationship of full-length GvpA of non-actinomycetes and the N termini of actinomycete GvpA. The phylogenetic tree was constructed using the Treecon v 1.3b program [23] inferred from alignments created by the ClustalX program [24]. Phylogenetic distances were determined by neighbour-joining analysis; numbers on branching points are bootstrap values with 100 replicates.

indicates that *gvp* sequences must have been present in a common actinomycete ancestor.

Are gas vesicle proteins required for flotation in actinomycetes?

The habitats of saprophytic actinomycetes range from soils to sediments; more recently, actinomycetes have also been discovered in marine environments [13].

Recently, it was shown that the non-motile *S. coelicolor* forms floating colonies in standing liquid cultures [14], a condition that resembles flooded, water-logged soils. These environments readily become anoxic; therefore, flotation and subsequent sporulation at the air–water interface provides an excellent escape from the unfavourable, oxygen-poor environment. Surprisingly, however, *S. coelicolor* mutants lacking both *gvp* clusters still floated and reached the air interface in standing liquid cultures (G. van Keulen, *et al.*, unpublished). This indicates that gas vesicle proteins, including the intriguing C-terminal extension on GvpA in actinomycetes, are not essential for flotation and suggests the existence of other, unknown mechanism(s) of buoyancy in addition to other roles for the Gvp proteins: what could they be?

Involvement of actinomycete Gvp proteins in other cellular processes

Transcript profiling has indicated that *gvp1* and neighbouring genes are transiently induced in expression in shaken liquid cultures of *S. coelicolor* after osmotic and temperature upshifts [15]. Recent evidence indicates that osmoadaptation is also important in erection of aerial hyphae and secondary metabolite production [16–18]. Whether the properties conferred by the C-terminal extension on GvpA have a role in actinomycete stress response or in differentiation remains to be established. One possibility is that the arginine-, glutamate- and proline-rich regions of GvpA, which are also found in GvpY and GvpZ, might have a role in binding of these Gvp proteins to nucleic acids. Similarly charged amino acid tracts are observed in virus coat proteins and RNA-binding proteins [19]. Furthermore, arginine-rich peptides have also been reported to assist the translocation of other peptides through membranes [20], which presents a further putative function for GvpA.

Concluding remarks

Until recently, the ability to synthesize gas vesicles was thought to be restricted to the aquatic cyanobacteria and the halophilic archaea [1,21]. A few other sporadic occurrences of *gvp* genes have been reported for soil bacteria, for example, *Bacillus megaterium* [22] and *Rhodobacter sphaeroides* (http://genome.jgi-psf.org/mic_home.html). Although gas vesicles have not been observed in these microorganisms, the *gvp* genes of *B. megaterium* can be expressed functionally in *Escherichia coli*, rendering it buoyant. Analysis of emerging genome sequences has revealed that many genera of free-living actinomycetes carry *gvp* genes as an apparently long-established and stable part of their genomes, even though actual gas vesicles have not been seen. It will be of interest to determine the function of Gvp proteins in the actinomycetes, and in particular to determine whether the long C-terminal extension confers roles other than in flotation.

It is notable that the actinomycetes that have *gvp* gene clusters generally have large genomes. Whether there is a correlation between genome size and the presence of *gvp* genes remains to be determined. It seems more likely that the absence of *gvp* genes from pathogenic mycobacteria and corynebacteria reflects their roles as specialized parasites rather than free-living saprophytes. It is unlikely that filamentous growth is the significant factor in actinomycetes with *gvp* genes because the *Thermobifida fusca* genome does not have *gvp* genes (http://genome.jgi-psf.org/mic_home.html).

Does the different composition of *gvp* clusters in the various actinomycetes perhaps suggest different functions for these multiprotein assemblies in these strains? And why do streptomycetes have more than one *gvp* gene cluster? These are exciting questions that need to be addressed to understand the role of the gas vesicle proteins in the complex biology of these microbes.

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